REMARKS

I. Status of the claims

Claims 1-25, 84, and 86-92, have been cancelled without prejudice or disclaimer. Of course, Applicants reserve the right to file one or more continuing applications to the cancelled subject matter. Claims 93-139 have been added.

The subject matter of new claims 93-139 further define the subject matter of originally-filed claims 61, 62, and 86-92. Pages 62-70 of the specification further support the newly-claimed subject matter, as do Examples 87-103 and figures 30, 31, 58, and 60. Accordingly, the new claims are fully supported by the present application and, hence, introduce no new matter. Applicants therefore request that the Examiner enter and consider claims 93-139.

II. Summary of the Present Invention

A key aspect of the presently claimed invention is Applicants' surprising discovery that a centromere of human chromosome #14 or of chromosome #21 imparts relative stability to a chromosome in a host cell. Thus, Applicants realized that a fragment of a chromosome containing such a human centromere could be used as a vector to carry and stably maintain in a cell at least one other chromosome fragment, such as a fragment embodying an antibody gene locus.

To this end, the present invention is directed to methods, compositions, and tools for modifying a chromosome, to produce a "recombinant chromosome," useful in producing a cell that stably contains a desired chromosomal fragment and expresses a desired gene on that fragment. More specifically, claims 93-139 are directed to a recombinant chromosome that comprises (i) the centromere of a human chromosome #14 or #21; (ii) two telomere sequences; (iii) at least one recognition sequence for site-directed recombination enzyme; (iv) at least two chromosome fragments that are not located adjacent to one another in their natural chromosomal environment; and (iv) a marker gene. For instance, a recombinant chromosome of the present invention may

contain the centromere of human chromosome #14, two telomeres, a loxP site, a fragment of human chromosome #22, and a marker gene (see claim 97).

III. Summary of the Office Action

The Examiner objected to the misspelling of "plurality" in claim 14; rejected claims 1-25, and 84, under 35 U.S.C. § 112, first paragraph; claims 1, 2, 10, 16, 19, 24, 25, 84, 86, and 88, under 35 U.S.C. § 112, second paragraph; claims 1-19, 21-23, 25, 84, 86-92, under 35 U.S.C. § 102(b) as anticipated by Tomizuka *et al.*, Nature Genetics, 16: 133-143, 1997; claims 1-6, 19, 21-23, and 25 under 35 U.S.C. § 102(b) as anticipated by Kuncherlapati *et al.* (WO 94/02602); claims 1-23, 25, 84, 86-92, as obvious under 35 U.S.C. § 103(a) in light of Tomizuka *et al.*, in view of Hadlaczky *et al.* (U.S. Patent No. 6, 025, 155); and claims 1-19, 21-25, 84, and 86-92 as obvious under 35 U.S.C. § 103(a) in light of Tomizuka *et al.*, in view of Gerdes, FEBS, 389:44-47, 1996.

IV. Applicants' amendment overcomes the Examiner's objection and rejections under 35 U.S.C. § 112, first and second paragraphs

Since Applicants have cancelled claims 1-25, 84, and 86-92, the Examiner's rejections are now moot, and Applicants respectfully request that the Examiner withdraw these rejections.

V. Claims 1-19, 21-23, 25, 84, 86-92, are not anticipated by Tomizuka *et al.*

The Examiner alleges that Tomizuka *et al.* teaches "the introduction of human chromosome or chromosome fragments into mouse ES cells by microcell-mediated chromosome transfer," where the chromosomal DNA carries "the genes for human antibodies from unrearranged human Ig genes, which are found on human chromosomes 2, 14 and 22, into mouse ES cells." Office Action at pages 8 and 9.

The Examiner further asserts that Tomizuka's experiments "would allow for the generation of mice containing any desired human chromosome or fragments derived from a specific chromosomal region," and that Tomizuka *et al.* teaches the use of the "Cre-loxP system to replace specific mouse chromosomal regions with the corresponding human

chromosomal fragment . . . by homologous recombination." *Id.* at page 9. According to the Examiner, since Tomizuka *et al.* uses "intact chromosomes," which necessarily would "have a telomere sequence," then Tomizuka *et al.* teaches the claimed targeting vector, which contains a telomere sequence." *Id.*

Without acquiescing to the Examiner's stated rationale, Applicants have cancelled the rejected claims, rendering the rejection moot. Accordingly, Applicants request that the Examiner withdraw the rejection.

Applicants also note that Tomizuka *et al.* discloses the introduction of a ***spontaneously fragmented*** human chromosome #14 fragment, or a full-length human chromosome, into mouse ES cells via microcell-mediated chromosome transfer. By contrast, Applicants' methodology relates to an ***artificially modified***, recombinant chromosome that contains a centromere of either human chromosome #14 or human chromosome #21, a recognition sequence for site-specific recombination enzyme, and at least one other chromosome fragment, all of which elements are integrated via chromosomal recombination and chromosomal engineering.

Furthermore, Tomizuka *et al.* employs the Cre-loxP system to remove a marker gene. Tomizuka *et al.* does not use the Cre-loxP system in a method prescribed by the present claims. That is, Tomizuka *et al.* does not use Cre-loxP-induced recombination to join together separate chromosomal fragments.

VI. Claims 1-6, 19, 21-23, and 25, are not anticipated by Kuncherlapati *et al.*

The Examiner alleges that Kuncherlapati *et al.* teaches "a method of producing mouse ES cells which have nonfunctional endogenous immunoglobulin genes, and have been introduced with xenogeneic, e.g., human heavy and light chain immunoglobulin genes . . . in particular, they teach that a yeast artificial chromosome (YAC) can be introduced into ES cells by oocytes by fusion cell [sic] with a yeast spheroplast . . . they further teach that a marker gene can be used in the targeting construct, such as G4128 resistance," Office Action at page 10. The Examiner also states that "the YAC containing yeast spheroplast as taught by Kuncherlapati is encompassed by the [present] specification's definition of a microcell," Office Action at page 10.

Since claims 1-6, 19, 21-23, and 25 have been cancelled, the Examiner's rejection is rendered moot, and Applicants respectfully request that the Examiner withdraw the rejection.

VII. Claims 1-23, 25, 84, and 86-92, are not rendered obvious by Tomizuka *et al.*, in view of Hadlaczky *et al.* (U.S. Patent No. 6, 025, 155)

The Examiner alleges that "in view of the combined teachings of Tomizuka and Hadlaczky, it would have been obvious . . . to modify the method of Tomizuka for producing cells comprising modified foreign chromosomes by microcell fusion followed by homologous recombination using a cell such as the chicken DT-40 cell," Office Action at page 12. According to the Examiner, the skilled artisan "would have been sufficiently motivated to make such a modification, as asserted by Hadlaczky, that DT-40 cells could be used in microcell fusion to introduce artificial chromosomes into avian cells," Office Action at page 12.

Applicants note that Hadlaczky says nothing of a recombinant chromosome comprising a centromere from either human chromosome 14 or chromosome 21, which is a vector for shuttling, and stably maintaining, a chromosome fragment into a host cell, as prescribed by the present claims.

Furthermore, since Applicants have cancelled claims 1-23, 25, 84, and 86-92, the Examiner's rejection is moot, and Applicants respectfully request that the Examiner withdraw the rejection.

VII. Claims 1-19, 21-25, 84, and 86-92 are not rendered obvious by Tomizuka *et al.*, in view of Gerdes, FEBS, 389:44-47, 1996

The Examiner acknowledges that Tomizuka *et al.* does not teach that "the marker gene that is used for screening the cells comprising foreign chromosomes is a green fluorescent protein encoding gene." Office Action at page 13. Even so, the Examiner asserts that the present claims are rendered obvious because Gerdes teaches that green fluorescent protein is useful "as a reporter for gene expression, a marker to study cell lineage, and as a tag to localize proteins in living cells." Office Action at page 13.

Without acquiescing to the Examiner's stated rationale, Applicants have cancelled the rejected claims, rendering the rejection moot. Accordingly, Applicants request that the Examiner withdraw the rejection.

VIII. Conclusion

In view of the above remarks and amendments, it is respectfully submitted that this application is in condition for allowance. Early notice to that effect is earnestly solicited. The Examiner is invited to telephone the undersigned at the number listed below if the Examiner believes such would be helpful in advancing the application to issue.

Respectfully submitted,

Date 22 April 2003

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